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Acclimation of *Nannochloropsis gaditana* to different illumination regimes: Effects on lipids accumulation

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ABSTRACT

Algae are interesting potential sources of biodiesel, although research is still needed to develop efficient large scale productions. One major factor affecting productivity is light use efficiency. The effect of different light regimes on the seawater alga *Nannochloropsis gaditana* was accessed monitoring growth rate and photosynthetic performances. *N. gaditana* showed the capacity of acclimating to different light intensities, optimizing its photosynthetic apparatus to illumination. Thanks to this response, *N. gaditana* maintained similar growth rates under a wide range of irradiances, suggesting that this organism is a valuable candidate for outdoor productions in variable conditions. In the conditions tested here, without external CO_2 supply, light intensity alone was not found to be a major signal affecting lipids accumulation showing the absence of a direct regulatory link between the light stress and lipids accumulation. Strong illumination can nevertheless indirectly influences lipid accumulation if combined with other stresses or in the presence of excess CO_2.

1. Introduction

Microalgae are receiving a growing attention for their possible exploitation for biofuels production. In fact, several species of this group are able to accumulate large amounts of lipids and can be a suitable feedstock for biodiesel production. Currently biodiesel is obtained from oil rich seeds, but this process, due to the feedstock limitation, has little chance to be able to replace a significant fraction of fossil fuels (Singh et al., 2011). In the case of algae, productivities are estimated to be around ten times the one of crops, making these organisms a more promising source for biomass production on a long term perspective (Chisti, 2007, 2008). Algae are a group of organisms with a very large biological variability and thus with very different properties. Among them, the ones belonging to the genus *Nannochloropsis* are particularly interesting because of their ability to accumulate large amounts of lipids, which can reach concentrations up to 65–70% of total dry weight (Boussiba et al., 1987; Hodgson et al., 1991; Rodolfi et al., 2009). Such a massive accumulation of lipids was shown to be activated during the stationary phase of growth in response to stresses such as nitrogen and/or phosphorous starvation (Gouveia and Oliveira, 2009; Rodolfi et al., 2009).

In order to exploit these organisms for large scale biofuel production, it is fundamental to optimize productivity by obtaining a better understanding of the parameters influencing growth, biomass and lipids accumulation. As for all photosynthetic organisms, light is a major factor to be considered as it provides all the energy supporting metabolism. Radiation, however, can also be dangerous because, if in excess, it may drive the formation of reactive oxygen species (ROS) and oxidative stress (Li et al., 2009). When cells are exposed to illumination, one component of the photosynthetic apparatus, photosystem II (PSII), is continuously damaged and inactivated in a well known process called photoinhibition (Murata et al., 2007). In order to maintain photosynthetic efficiency, this complex must be continuously repaired by re-synthesis of damaged components (Nixon et al., 2010). Besides repairing the photoinhibitory damage, photosynthetic organisms have also evolved mechanisms to optimize light harvesting efficiency to physiological needs so as to prevent ROS formation and photoinhibition. Among these is light acclimation, where the composition of the photosynthetic apparatus is optimized according to irradiance intensity (Falkowski and LaRoche, 1991; Falkowski and Owens, 1980; Walters, 2005; Zou and Richmond, 2000). Photoinhibition and its repairing is energetically very demanding and may strongly influence the biomass productivity. The capacity for an effective

Abbreviations: Car, carotenoid; Chl, chlorophyll; NPQ, non photochemical quenching; NR, Nile Red; PS, photosystem; qP, photochemical quenching; ROS, reactive oxygen species.

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and prompt acclimation is therefore an important parameter to be considered in the characterization of an algal species for biomass production. In addition to its metabolic role, light is also known to play a large effect in signaling and its intensity affects many cellular processes (Brautigam et al., 2009; Eberhard et al., 2008; Kim et al., 2008).

The light intensity has also been suggested to influence algae capacity for lipids accumulation, which would be enhanced under strong illumination (Damiani et al., 2010). Such a phenomenon has been observed also for *Nannochloropsis* species, where a transition from control to high light conditions was found to induce a higher level of lipids accumulation (Fisher et al., 1998; Solovchenko et al., 2010).

In this work, the acclimation capacity of *Nannochloropsis gaditana* to different light regimes was analyzed, showing that during the exponential growth phase this organism is able to adapt to a wide range of light intensities by activating an acclimative response, as evidenced by the functional characterization of its photosynthetic apparatus. The light intensity *per se* does not appear to directly induce lipids accumulation, but excess illumination may enhance the effect of other stresses.

2. Methods

2.1. Culture conditions

N. gaditana from CCAP, strain 849/5, was always grown in sterile filtered F/2 medium (Guillard and Ryther, 1962), using sea salts 32 g/l from SIGMA, 40 mM TRIS HCl pH 8, SIGMA Guillard's (f/2) marine water enrichment solution 1×. Maintenance and propagation of cultures were performed using the same medium added with 10 g/l of Plant Agar (Duchefa Biochemie). Growth experiments were performed in Erlenmeyer flasks with magnetic agitation, starting from a pre-culture grown at 100 $\mu E\ m^{-2}\ s^{-1}$ at exponential phase which was diluted to an optical density (OD) value at 750 nm equal to 0.2, final volume 250 ml. Illumination was constant and intensities ranged from 5 to 2100 $\mu E\ m^{-2}\ s^{-1}$, using daylight fluorescent lamps (5–200 $\mu E\ m^{-2}\ s^{-1}$) or a LED Light Source SL 3500 (Photon Systems Instruments, 450–2100 $\mu E\ m^{-2}\ s^{-1}$). Temperature was kept at 23 ± 1 °C in a growth chamber. No external CO_2 supply was provided and thus carbon dioxide to support growth derived from the atmosphere. Algal growth was measured by daily changes in optical density OD_{750} determined spectrophotometrically with a Lambda Bio 40 UV/VIS Spectrometer (Perkin Elmer) and cells number monitored with a Bürker Counting Chamber (HBG, Germany) under light microscope. In the logarithmic growth phase cells number was related to optical density. The specific growth rate was calculated by the slope of logarithmic phase for number of cells. All curves were repeated at least three times and in all repetitions one culture at 100 $\mu E\ m^{-2}\ s^{-1}$ was always present as control.

2.2. Pigments extraction and analysis

Chlorophyll a and total Car were extracted from *Nannochloropsis* centrifuged cells at 4 °C with 100% *N,N′*-dimethylformamide for at least 48 h in dark conditions as in (Moran and Porath, 1980). The pigments concentrations were determined spectrophotometrically using specific extinction coefficients (Porra et al., 1989; Wellburn, 1994).

2.3. In vivo monitoring of photosynthetic parameters

Chlorophyll fluorescence was determined in vivo using Dual PAM 100 from WALZ. The parameters Fv/Fm, NPQ and qP were calculated respectively as (Fm–Fo)/Fo, (Fm–Fm′)/Fm′ and (Fm′–F)/(Fm′–Fo) (Demmig-Adams et al., 1996) after 20 min of dark adaptation.

2.3.1. RuBIsCO content analysis

SDS–PAGE analysis was performed with a tris–glycine buffer system as in (Laemmli, 1970) with 12% acrylamide.

2.4. Lipids analysis

The lipid content was determined by staining 2 millions of algal cells centrifuged and resuspended in 1.9 ml of de-ionized sterile water with Nile Red (NR) dye at final concentration of 2.5 μg/mL, for 10 min at 37 °C (Chen et al., 2009). The fluorescence was measured using a spectrofluorometer (OLIS DM45), with excitation wavelength at 488 nm and emission wavelength in the range of 500 and 700 nm (Greenspan et al., 1985). The relative fluorescence of Nile Red for the lipids was obtained after subtraction of the autofluorescence of algal cells and Nile Red alone. Total lipids were extracted from dried cells using ethanol–hexane (2.5:1 vol/vol) as solvent in a Soxhlet apparatus for 10 h. The lipid mass was measured gravimetrically after solvent removal by a rotary evaporator. The fluorescence intensity of cells stained by NR is linearly correlated to the gravimetric ratio of cellular lipid.

3. Results and discussion

3.1. Effect of different irradiances on N. gaditana growth

Light has multiple effects on photosynthetic organisms, it provides the energy to support metabolism but it is also a fundamental signal influencing many cellular processes. In order to investigate light influence on *Nannochloropsis* growth and lipids accumulation one preliminary choice to be made was if providing externally carbon dioxide in excess or rely on the smaller amount present in the atmosphere. In the former case photosynthesis rate is increased while the latter is closer to environmental conditions. The aim of the work was not to achieve the maximal productivity but to investigate the regulatory connection between light intensity and the activation of lipid biosynthesis. In order to analyze regulation effects the conditions closer to a natural environment were chosen as more significant and thus experiments were performed with atmospheric CO_2 concentration.

N. gaditana was grown in batch cultures exposed to a wide interval of light conditions, ranging from very low to very high, respectively 5, 15, 50, 100, 200, 450, 1200, 2100 $\mu E\ m^{-2}\ s^{-1}$. In all cultures growth kinetic was monitored following the increase in cells number, as shown in Fig. 1. Data presented show that growth rates are remarkably similar for most light intensities tested and significant differences are observed only for the extreme conditions (5, 1200 and 2100 $\mu E\ m^{-2}\ s^{-1}$). In particular, cultures exposed to very low light showed slower growth, clearly imputable to a limitation in the energy available to support metabolism. On the other extreme, cultures exposed to very strong light reached the stationary phase earlier and final cells number was lower. The specific growth rate (μ) has very similar values for all curves but in the case of that at 5 $\mu E\ m^{-2}\ s^{-1}$ (Fig. 1D). Growth rates are similar to control also in the case of cultures exposed to the highest illumination (1200 and 2100 $\mu E\ m^{-2}\ s^{-1}$), although in this case experimental deviation is higher because values are calculated with a lower number of data points because of a shorter exponential phase.

Such a small effect of illumination on growth kinetics suggests that in these conditions, the duplication rate of *N. gaditana* is not light limited but depends on other factors like CO_2 and nutrient